Symposium: Flagella and Motility

2735-Symp
Flagellar Motor Remodeling and Filament Growth
Howard Berg.
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As symposium chair, I will tell you a few things about bacterial motility that *E. coli* knows, and then describe two recent vignettes, involving adaptation at the output of the sensory-transduction pathway, and growth of flagellar filaments. 1) Receptor methylation and demethylation are required for adaptation on the second time scale, which enables cells to make temporal comparisons and swim up spatial gradients of attractants. In the absence of the methyltransferase and the methylesterse, one still observes partial adaptation, on the minute time scale. The motor shifts its operating point to accommodate new steady-state levels of the response regulator CheY-P. When the concentration of CheY-P decreases, the motor spins CCW and the number of copies of the protein to which CheY-P binds, FliM, increases, partially restoring motor sensitivity. 2) Flagellar filaments grow at their distal ends. The dogma in the field asserts that they do so at a rate that decreases exponentially with length. By labeling filaments twice, first with a green fluorescent dye and later with a red fluorescent dye, we find that filaments grow at a constant rate. On average, the lengths of red segments do not depend upon the lengths of green segments from which they grew.

2736-Symp
Structural Insight into Torque Generation Mechanism of the Bacterial Flagellar Motor
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The bacterial flagellum is made of a rotary motor and a long helical filament by means of which bacteria swim. The flagellar motors of Salmonella and E. coli rotate at around 300 Hz and drive rapid rotation of each flagellum to propel cell movements. The mechanism of torque generation by the motor has been intensively studied by many groups based on the torque-speed relationships and the structure, but it has been elusive due to the lack of high-resolution structural information of the rotor and stator and their interactions. X-ray crystal structures are available for some part of the rotor and switch complex component proteins but no detailed information is yet available for their arrangement in the motor assembly and also for the transmembrane and cytoplasmic domains of the stator. A currently proposed 3D model of the rotor for torque generation does not satisfy the stepping behavior of the motor observed by single motor nanophotometry. We have been trying to solve the structure of the flagellar hook-basal body by electron cryomicroscopy and single particle image analysis in its isolated form from the membrane as well as by electron cryotomography of the cell to visualize the basal body in situ. I will report our current progress and discuss some structural insights into the torque generation mechanism.

2737-Symp
Assembly and Function of the Archaeal Motility Structure, the Archaellum
Sonja-Verena Albers.
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Most archaea posses a motility structure, called the archaellum that is mainly used for swimming, but can also play a role in adherence to surfaces. Although the archaellum functionally resembles the bacterial flagellum, its structure and assembly is reminiscent of bacterial type IV pili.
We use the crenarchaeal archaellum of Sulfolobus acidocaldarius as a model system to understand its assembly, subunit interactions and how it can rotate. The crenarchaeal archaellum is comprised of seven subunits: the archaellin, FlaB, the structural subunit, FlaXFG, small monotopic membrane proteins of unknown function, FlaH, an ATP-binding protein, FlaI, the motor ATPase and FlaJ, the only polytopic membrane protein.
We showed that FlaI forms an ATP-dependent hexamer and its N-terminus is involved in the switch between the rotation and assembly of the filament. FlaH is a typical RecA like protein which can only bind ATP, but not hydrolyze it. Its function in motor rotation or assembly is so far unknown. FlaX, a conserved crenarchaeal archaellum protein, formed a large ring structure with a diameter of 22 nm and interacted with FlaH and FlaI, presumably acting as a stator like structure.
Biophysical studies like tethered motion particle analysis and optical tweezer experiments are being used to understand the mode of rotation of the archaellum and will be presented.

2738-Symp
Structure-Based Analysis of the Type III Secretion System for Antimicrobial and Vaccine Design
Natalie Strynadka.
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Bacteria have evolved several dedicated and sophisticated assemblies to transport proteins across their biological membranes. Recent advances in our understanding of the molecular details governing the specific actions of these protein secretion systems has come from an integrated approach of x-ray crystallography, NMR, mass spectroscopy, electron microscopy and in vitro reconstitution/in vivo phenotypic analysis. Highlights of recent advances will be presented with an emphasis on that of the Type III Secretion system, the so-called bacterial injectisome encoded exclusively by pathogenic Gram negative strains including *Salmonella typhimurium*, *Yersinia pestis, Pseudomonas aeruginosa* and *enteropathogenic Escherichia coli*. A structure-based and genetic piecing together of the Type III Secretion System indicates that more than two dozen proteins assemble into a large needle shaped complex spanning the inner and outer bacterial membranes as well as that of the infected host cell, providing a direct conduit for the transport of essential bacterial virulence effectors from bacterial to host cytosol. A molecular understanding of the Type III secretion system being garnered from these studies provides the foundation for the development of new classes of vaccines and antimicrobials to combat these pathogens in the clinic and community.

Symposium: Biological Circuit Design

2739-Symp
Synthetic Biology: From Parts to Modules to Therapeutic Systems
Ron Weiss.
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Synthetic biology is revolutionizing how we conceptualize and approach the engineering of biological systems. Recent advances in the field are allowing us to expand beyond the construction and analysis of small gene networks towards the implementation of complex multicellular systems with a variety of applications. In this talk I will describe our integrated computational / experimental approach to engineering complex behavior in living systems ranging from bacteria to stem cells. In our research, we appropriate design principles from electrical engineering and other established fields. These principles include abstraction, standardization, modularity, and computer aided design. But we also spend considerable effort towards understanding what makes synthetic biology different from all other existing engineering disciplines and discovering new design and construction rules that are effective for this unique discipline. We will briefly describe the implementation of genetic circuits and modules with finely-tuned digital and analog behavior and the use of artificial cell-cell communication to coordinate the behavior of cell populations. The first system to be presented is an RNAi-based logic circuit that can detect and destroy specific cancer cells based on their microRNA expression profiles. We will also discuss preliminary experimental results for obtaining precise spatiotemporal control over stem cell differentiation for tissue engineering applications. We will conclude by discussing the design and preliminary results for creating an artificial tissue homeostasis system where genetically engineered stem cells maintain indefinitely a desired level of pancreatic beta cells despite attacks by the autoimmune response, relevant for diabetes.

2740-Symp
Noise Suppression and Pulse Generation in a Microrna Feed-Forward Loop
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MicroRNA have long been known to aid in providing tissue identity or in canalizing development, roles that require the long-term stable maintenance of regulatory states. In particular, it has been suggested that a key role of microRNA is to help reduce noise in the expression of their target genes. However, so far, there has been relatively limited quantitative experimental support for this hypothesis. using synthetic regulatory networks stably integrated in a mammalian cell line, we recently showed that microRNA can indeed dramatically reduce noise due to variation in an upstream regulator but that this effect is highly dependent on the regulatory context; noise suppression was most effective if the miRNA was co-regulated with its target in an incoherent feed-forward loop (IFFL). In an IFFL an upstream transcription factor activates the expression of a downstream target gene while also, counter-intuitively, activating a negative regulator — in this case a miRNA — that represses that same target gene.